

CLAIMS

We claim:

1. A method for the design and synthesis of domain-selective inhibitors of angiotensin-converting enzyme (ACE) that are highly selective for either the N domain or the C domain of ACE, by the use of the three-dimensional crystal structure of an angiotensin-converting enzyme, comprising the steps of:
 - a. using a three-dimensional structure of said enzyme as defined by atomic coordinates of an angiotensin-converting enzyme;
 - b. employing said three-dimensional structure to design or select said inhibitors;
 - c. synthesizing said inhibitors;
 - d. contacting said N- and C-domain-selective inhibitors with said enzyme in the presence of a substrate to determine the ability of said inhibitors to selectively inhibit the N and C domains, respectively, of ACE; and
 - e. co-crystallizing said N- and C-domain-selective inhibitors with said enzyme to determine and optimize the ability of said inhibitors to selectively inhibit the N and C domains, respectively, of ACE.
2. A method of claim 1 wherein the three-dimensional crystal structure of ACE is of the full-length, wild-type somatic form of the enzyme, generated recombinantly in CHO cells, COS cells, or other suitable mammalian cells, or other eukaryotic, e.g., yeast, or prokaryotic, e.g., *Escherichia coli*, cells, or purified from natural sources, such as lung or kidney tissue.
3. A method of claim 1 wherein the three-dimensional crystal structure of ACE is of the full-length, wild-type testis form of the enzyme, generated recombinantly in CHO cells, COS cells, or other suitable mammalian cells, or other eukaryotic, e.g., yeast, or prokaryotic, e.g., *Escherichia coli*, cells, or purified from natural sources, such as testis tissue.

4. A method of claim 1 wherein the three-dimensional crystal structure of ACE is of the isolated N domain of the somatic form of the enzyme, generated recombinantly in CHO cells, COS cells, or other suitable mammalian cells, or other eukaryotic, e.g., yeast, or prokaryotic, e.g., *Escherichia coli*, cells, or generated by limited proteolysis of somatic ACE purified from natural sources, such as lung or kidney tissue, or generated by peptide synthesis.
5. A method of claim 1 wherein the three-dimensional crystal structure of ACE is of the isolated C domain of the somatic or testis forms of the enzyme, generated recombinantly in CHO cells, COS cells, or other suitable mammalian cells, or other eukaryotic, e.g., yeast, or prokaryotic, e.g., *Escherichia coli*, cells, or generated by limited proteolysis of somatic or testis ACE purified from natural sources, such as lung, kidney, or testis tissue, or generated by peptide synthesis.
6. A method of claim 1 wherein said angiotensin-converting enzymes contain one or more site-specific or regional mutations, deletions, truncations, insertions, glycosylation changes, or other modifications that facilitate or enhance protein expression, purification, crystallization, x-ray diffraction, or x-ray structure determination or refinement.
7. A method of claim 1 wherein said N- and C-domain-selective inhibitors are designed and synthesized de novo.
8. A method of claim 1 wherein said N- and C-domain-selective inhibitors are designed and synthesized from one or more known inhibitors.
9. A method of claim 1 wherein said N- or C-domain-selective inhibitors are competitive inhibitors of angiotensin-converting enzyme.
10. A method of claim 1 wherein said N- or C-domain-selective inhibitors are non-competitive, uncompetitive, or irreversible inhibitors of angiotensin-converting enzyme.

11. A method for the design and synthesis of inhibitors of angiotensin-converting enzyme (ACE) that are non-selective and active against both the N domain and the C domain of ACE, by the use of the three-dimensional crystal structure of an angiotensin-converting enzyme, comprising the steps of:
- a. using a three-dimensional structure of said enzyme as defined by atomic coordinates of an angiotensin-converting enzyme;
 - b. employing said three-dimensional structure to design or select said inhibitors;
 - c. synthesizing said inhibitors;
 - d. contacting said non-selective inhibitors with said enzyme in the presence of a substrate to determine the ability of said inhibitors to inhibit both the N and C domains, respectively, of ACE; and
 - e. co-crystallizing said non-selective inhibitors with said enzyme to determine and optimize the ability of said inhibitors to inhibit both the N and C domains, respectively, of ACE.
12. A method of claim 11 wherein the three-dimensional crystal structure of ACE is of the full-length, wild-type somatic form of the enzyme, generated recombinantly in CHO cells, COS cells, or other suitable mammalian cells, or other eukaryotic, e.g., yeast, or prokaryotic, e.g., *Escherichia coli*, cells, or purified from natural sources, such as lung or kidney tissue.
13. A method of claim 11 wherein the three-dimensional crystal structure of ACE is of the full-length, wild-type testis form of the enzyme, generated recombinantly in CHO cells, COS cells, or other suitable mammalian cells, or other eukaryotic, e.g., yeast, or prokaryotic, e.g., *Escherichia coli*, cells, or purified from natural sources, such as testis tissue.
14. A method of claim 11 wherein the three-dimensional crystal structure of ACE is of the isolated N domain of the somatic form of the enzyme, generated recombinantly in CHO cells, COS cells, or other suitable mammalian cells, or other eukaryotic, e.g., yeast, or prokaryotic, e.g., *Escherichia coli*, cells, or generated by limited proteolysis of somatic

ACE purified from natural sources, such as lung or kidney tissue, or generated by peptide synthesis.

15. A method of claim 11 wherein the three-dimensional crystal structure of ACE is of the isolated C domain of the somatic or testis forms of the enzyme, generated recombinantly in CHO cells, COS cells, or other suitable mammalian cells, or other eukaryotic, e.g., yeast, or prokaryotic, e.g., *Escherichia coli*, cells, or generated by limited proteolysis of somatic or testis ACE purified from natural sources, such as lung, kidney, or testis tissue, or generated by peptide synthesis.

16. A method of claim 11 wherein said angiotensin-converting enzymes contain one or more site-specific or regional mutations, deletions, truncations, insertions, glycosylation changes, or other modifications that facilitate or enhance protein expression, purification, crystallization, x-ray diffraction, or x-ray structure determination or refinement.

17. A method of claim 11 wherein said non-selective inhibitors are designed and synthesized de novo.

18. A method of claim 11 wherein said non-selective inhibitors are designed and synthesized from one or more known inhibitors.

19. A method of claim 11 wherein said non-selective inhibitors are competitive inhibitors of angiotensin-converting enzyme.

20. A method of claim 11 wherein said non-selective inhibitors are non-competitive, uncompetitive, or irreversible inhibitors of angiotensin-converting enzyme.